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Interaction of active compounds from *Aegle marmelos* CORREA with histamine-1 receptor

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Abstract:
The aim of this study is to determine the affinity of six active compounds of *Aegle Marmelos* Correa, they are (E, R)-Marmin, skimmianine, (S)-aegeline, aurapten, zeorin, and dustanin as antihistamines in histamine H1 receptor in comparison to cetirizin, diphenhydramine and chlorpheniramine as ligands comparison. Previously, in the *in vitro* study marmin obviously antagonized the histamine H1 receptor in a competitive manner. Methods: *molecular docking* to determine the interaction of ligand binding to its receptor. Lower docking score indicates more stable binding to that protein. Results: Marmin, skimmianine, aegeline, aurapten, zeorin, and dustanin were potential to develop as antihistamine agents, especially as histamine H1 receptor antagonists by interacting with amino acid residues, Asp107, Lys179, Lys191, Asn198, and Trp428 of histamine H1 receptor. Conclusions: Based on molecular docking, Amino acid residues involved in ligand protein interactions were Asp107, Lys179, Lys191, Asn198, and Trp428.

Keywords: Marmin, Antihistamine, Ligand, Protein, Docking.

Background:
Narrowing of airways is a symptom of allergies caused by tracheal smooth muscle contractions. Histamine plays an important role in airway of smooth muscle contraction. Activation of histamine H1 receptors induce airway obstruction in human by histamine **[1]**. The therapies of allergies symptoms in respiratory tract can use the substances that inhibit the interaction of histamin with its receptors. This substance is named histamin receptor antagonists (antihistamins). The drugs have been used extensively to treat allergies. The mechanism of antihistamins is antagonising the histamin receptors, especially the H1 receptor **[2]**.

Various natural compounds isolated from plants have been developed and used as medicines to treat various diseases. Recently, the studies of new drug development from various plants are continuing such as marmin [(7 - (6 ', 7'-dihydroxygeranyl-oxy) coumarine], skimmianine [4,7,8 - (trimetoxyfuro (2.3 - b) quinolin], aegeline [N-[2-hydroxy-2 (4-metoxyfenyl) ethyl]-3-phenyl-2-propenamide], aurapten, zeorin, and dustanin. They are the active compounds contained in stem bark and cortex roots of *Aegle marmelos* Correa **[3-5]**. Reportedly, marmin is very potent to inhibit the histamin release from cultured RBL-2H3 cells through inhibition of Ca^{2+} uptake *in vitro*. Marmin at a concentration of 100 µM suppressed the release of histamin by more than 60% (which is induced by thapsigargin and DNP_{24}-BSA) and by more than 50% (which is induced by ionomycin) in comparison to those of the control group **[5]**. Marmin was also able to inhibit the influx of Ca^{2+} extracellular into RBL-2H3 cells, then inhibit the release of histamin by 70% in comparison to those of the control group **[6]**. Skimmianine at a concentration of 100 µM depleted the release of histamin by 60% (which are induced by DNP_{24}-BSA and thapsigargin) and by more than 70% (which is induced by ionomycin) in comparison to those of the control group **[3]**.

Methodology:

Collection of histamine protein sequence

The structure of protein as a target was downloaded from the Protein Data Bank website (http://www.rcsb.org/pdb). The protein was H1 histamine receptor (PDB code of 3RZE). As a ligand assay used six active compounds of *Aegle Marmelos* Correa, these were (E, R)-marmin, skimmianine, (S)-aegeline, auraptene, zeorine, and dustanine. For comparison of antihistamine, cetirizine, diphenhydramine and chlorpheniramine were used **Table 1 (see supplementary material).**

Protein Preparation

Homology modelling was accomplished by using YASARA program. FASTA files of 3RZE (the enzyme was downloaded from website: http/www.pdb.org).First, 3RZE pdb file inserted to the YASARA program. Removing part of the system was not required in docking protocol (merely require a single protein and ligand, including water if essential). Subsequently, 5EH should have removed that still appear in the system. Thus, Adding hydrogen into the system by YASARA program, since the resolution of crystal structures are not able to predict the presence of hydrogen. Adding hydrogen into a single protein has a result of **3RZE.yob**.Then, removing the original ligand, take only the target protein alone with a pocket for simulating docking. 3RZE.yob must be changed into **protein.mol2** because docking simulation only though that a pocked used to attach by ligand was a protein.

Ref_ligand Preparation

Ref_ligand preparation was done by using YASARA program. 3RZE.yob was needed to get a ref_ligand. Ref_ligand is helped a ligand finding coordinates pocket, so it was very important of simulation by molecular docking. Chemical compounds were on the 3RZE.yob must have to remove, except for 5EH and D7V. ref_ligand.mol2 was a result of 5EH and D7V by removing chemical compound that still exist on a protein.

Ligand Preparation

Three dimensional (3D) configuration structure and initial of ligand were contributed significantly in quality of molecular docking simulations. Differences in decision of protonation states and tautomers of the ligands for SBVS input can lead to different solutions of the SBVS protocols **[7, 8]**. Marvin of ChemAxon was chosen to be applied since the application provides features to perform protonation analysis in desired pH as well as tautomers and conformers generations **[9]**. ChemAxon also provides objective functions that enable us to assess and choose the most plausible tautomer and the 3D configurations of the ligand in appropriate protonation state **[9]**. (E, R)-marmine, skimmianine, (S)-aegeline, auraptene, zeorine, and dustanine were ligands for docking simulation. The results are deposited as a mol2 file to be applied further in molecular docking simulations using PLANTS **[8]**. Save as ligand.mol2.

Simulation Docking PLANTS

The active constituents that were docked with the protein are (E, R)-marmin, skimmianine, (S)-aegeline, auraptene, zeorine, and dustanine. After the docking process was finishing, you will see 10 of ligand conformation that binds to the 3RZE with different energies. For evaluation and outcome interpretation data of docking were chosen ten conformations which have the smallest score.

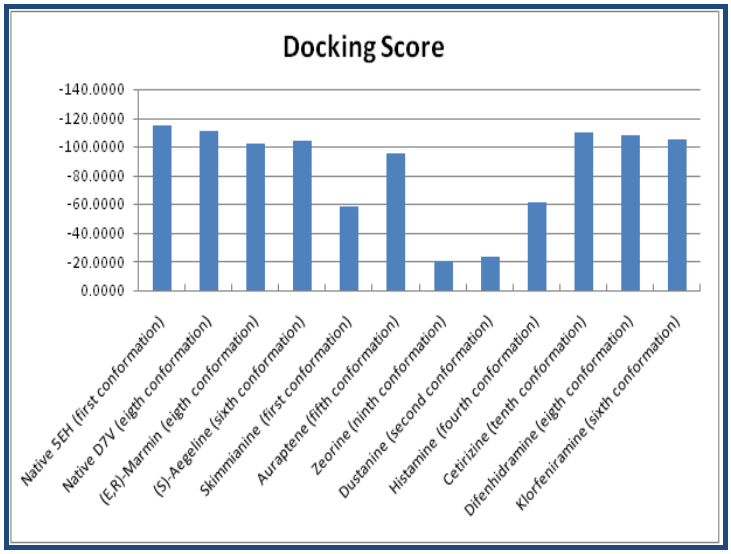

Figure 1: Docking score of ligand molecules test and ligand's comparison on 3RZE. Note : docking score that shown is scoring which obtained from ligand binding protein with the lowest energy

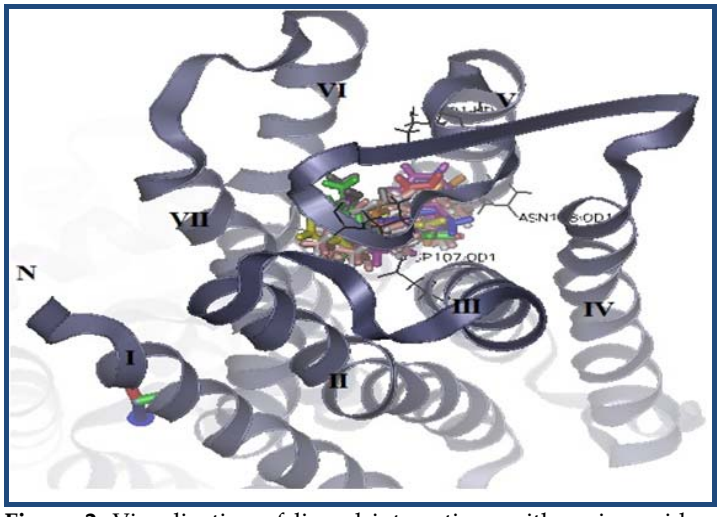

Figure 2: Visualization of ligand interactions with amino acid residues of H1 histamine receptor, *Asp107*, *Lys179*, *Lys191*, *Asn198*, and *Trp428*. Note: amino acid residues (black); *native ligand* of 5EH (blue); *native ligand* of D7V (red); (*E,R*)-marmin (gray); skimmianine (orange); (*S*)-aegeline (yellow); auraptene (green); zeorine (white); dustanine (pink); histamine (cyan); cetirizine (purple); diphenhydramine (green bright); chlorpheniramine (magenta)

Results & Discussion:

Docking protocol which used in this step like the docking's protocol validation step, there are docking each of 10 ligands conformation and ligands comparison test on the protein which has been prepared before. Based on the docking score, marmin compound, skimmianin, aegelin, cetirizin, diphenhydramine and chlorpheniramine were docked onto the histamine H1 receptor, and obtained the following results showed in **(Figure 1)**.

Based on results of visualization above **(Figure 2 & 3)** show that the ligands (marmin, skimmianine, aegeline, auraptene, zeorine, and dustanine) may interact with the amino acid residues, Asp107, Lys179, Lys191, Asn198, and Trp428 of the histamine H1 receptor including have different bond distances of each. Based on those interactions, the hydrogen bonds form and van der Waals forces were predicted. **Figure 2** shows that which amino acid residues dominantly play role in receptor-ligand interactions, they were in helix III, V, and VI. According to Shimamura *et al* (2011) and Rahim (2010) both of amino acids Asp107 and Trp428 were amino acid playing important role on binding of histamine receptor as antagonists, as well as in GPCR activation **[7, 10]**. When viewed from the distance of amino acid's interaction, native ligand, ligands, and ligand's comparison to Asp107 and Trp428, native ligand and ligand's comparison have more closer the bond distances (smaller energy).

In previous study, marmin was reported to be a very potent inhibitor of the histamine release from RBL-2H3 cell cultures by inhibition on Ca^{2+} uptake. Marmin at a concentration of 100 µM suppressed the release of histamine by more than 60% (which is induced by thapsigargin and DNP_{24}-BSA) and by more than 50% (which is induced by ionomycin) in comparison to the control **[5]**. Marmin also able to inhibit extracellular Ca^{2+} influx into RBL-2H3 cells, and inhibited the release of histamine by > 70% in comparition to the control **[7]**. Skimmianine at a concentration of 100 µM succeeded to deplete the release of histamine by 60% (which is induced by thapsigargin and DNP_{24}-BSA) and by more than 70% (which is induced by ionomycin) in comparison to the control cell **[3]**. Aegeline at a concentration of 100 µM suppressed the release of histamine by 40% (which is induced by DNP_{24}-BSA) and by more than 50% (which is induced by thapsigargin and ionomycin) in comparison to the control cell **[4]**. The results of Nugroho *et al*. study (2011) showed that Marmin has an activity as competitive reversible antagonist of H1 receptor, non-competitive reversible antagonist of ACh Mus3-receptor, and no effect on β2-adrenergic receptor. These results showed that marmin, skimmianine, and aegeline are potential to develop as an anti-allergic agent, especially as histamine H1 receptor antagonists (antihistamines H1) **[11]**.

Cetirizin is a second-generation of H1 antihistamines (second-generation antihistamines) that non-sedative antihistamine than another and selectively binds to the histamine H1 receptor, and also acts as an inverse agonist **[12]**. Diphenhydramine, also acts as an inverse agonist at the histamine H1 receptor **[10]**. Chlorpheniramine is a first-generation of antihistamines are widely used in the treatment of allergies, which competes with endogenous compound of histamine in histamine H1 receptor and also inhibits the action of endogenous histamine **[9]**. All of three antihistamines are used as a comparison to determine whether the active compounds of *Aegle Marmelos* Correa has an antihistamine effect.

Virtual screening demonstrated interaction between the ligand and amino acids of the protein histamine-1 receptor **[13]**. The histamin-1 receptor have many kinds of amino acids, however only spesific amino acids contributing to the histaminrgic activity, they are Asp107, Lys179, Lys191, Asn198, and Trp428. Based on the calculation of bond distance, cetirizin exhibited closer proximity distance of the amino acids Asp107, Lys191, and Trp428 than (E, R)-Marmin. Cetirizin has a distance of 1.72 angstrom on amino acids Asp107, 3:36 angstrom on amino acids Lys191, and 3:26 angstrom on Trp428. Whereas (E, R)-Marmin has distances of 2.69 angstrom; 3.85; and 4.97 angstrom respectively. The closest a ligand to the receptor indicates that the greatest intrinsic activity appeared. The proximity result of cetirizin has required less energy (-109.4690) than (E, R)-Marmin (-102.2860) for binding to the active site of histamine-1 receptor. However (E, R)-marmin still had antihistamin activity more higher than auraptene, histamin, skimmianine. Those results were not only based on the docking score, but based also on the interaction distance ligand to the active site of a protein. It was caused by the distance of auraptene, histamin, skimmianine were higher than that of (E, R)-marmin.

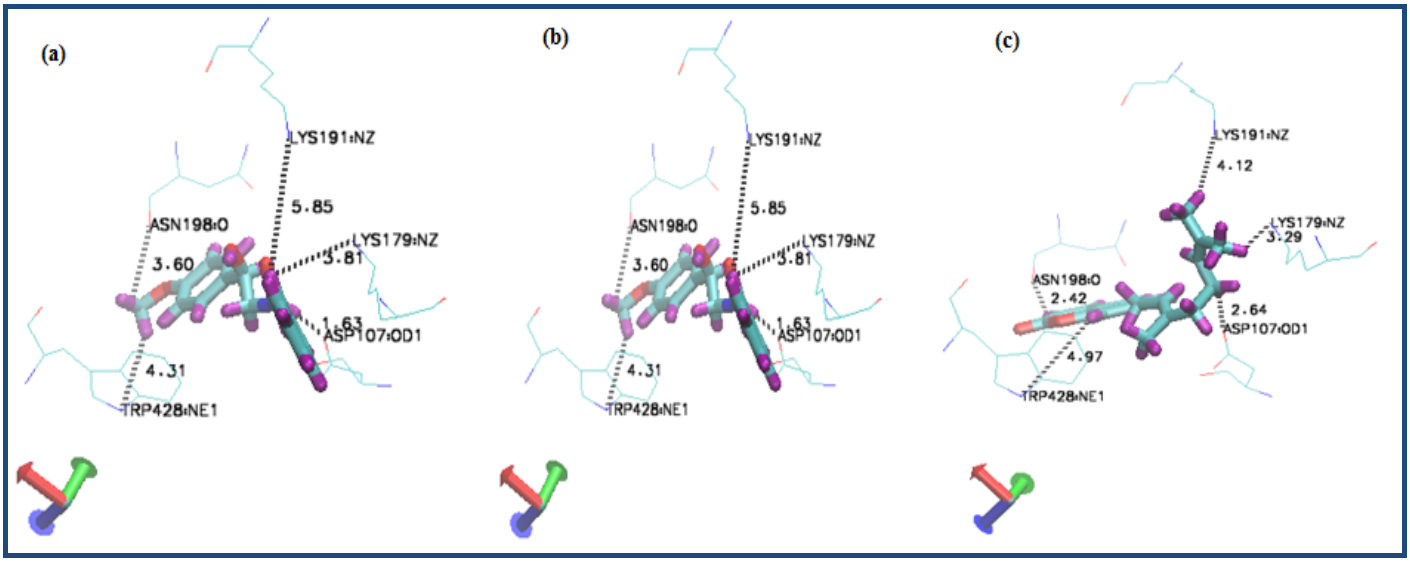

Figure 3: Visualization the distance of ligand interactions with amino acid residues of H1 histamine receptor *Asp107, Lys179, Lys191, Asn198*, and *Trp428*, respectively (a) (*E,R*)-marmin (b) (*S*)-aegeline (c) auraptene. Each ligand forming Hydrogen bonds (black dotted line) with active site residues depicted as line.

Conclusions:
Natural compounds with the best score on H1 antihistamines assay is native ligand 5EH. Based on molecular docking, Amino acid residues involved in ligand protein interactions were Asp107, Lys179, Lys191, Asn198, and Trp428.

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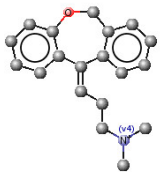
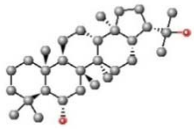
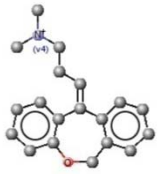
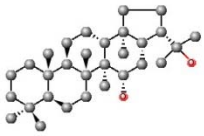
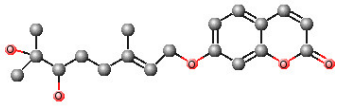
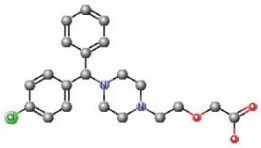
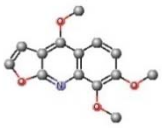
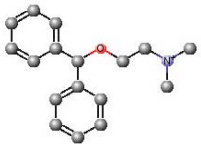
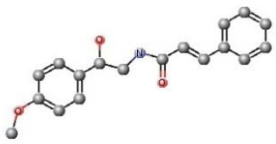
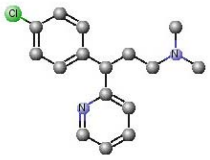
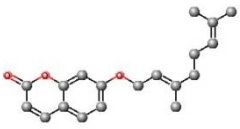
Supplementary material:

Table 1: The structure both of ligand molecules test and ligands comparison as antihistamin **(1)** *native ligand* 5EH **(2)** *native ligand* D7V **(3)** (*E,R*)-marmin **(4)** skimmianine **(5)** (*S*)-aegeline **(6)** auraptene **(7)** zeorine **(8)** dustanine **(9)** cetirizine **(10)** diphenhydramine **(11)** chlorpheniramine **(12)** histamine

No	Molecule	Structure	No	Molecule	Structure
1	*native ligand* 5EH		7	zeorine	
2	*native ligand* D7V		8	dustanine	
3	(*E,R*)-marmin		9	cetirizine	
4	skimmianine		10	diphenhydramine	
5	(*S*)-aegeline		11	chlorpheniramine	
6	auraptene		12	histamine